REMARKS

Claims 11-19 and 38-42 were being examined. Applicants cancelled claims 11-19, 39 and 41-42 without prejudice. Applicants amended claims 38 and 40 and added claims 43-44. Accordingly, claims 38, 40, 43-44 are now being examined.

The amendments to claims 38 and 40 and new claims 43-44 are supported by the specification as originally filed. For example, support for amendment to claim 38 can be found at page 9, lines 18-23, and support for amendment to claim 40 can be found at page 15, lines 8-11 of the specification.

Support for new claim 43 may be found in the specification at page 35, lines 1-4 and at page 36, line 31-35.

Support for new claim 44 may be found in the specification at page 47, lines 9-11 and at page 48, line 14.

Additionally, amendments to claims 38 and 40 are supported by Freeman et al. (J. Immunol. 143(8):2714-2722 (1989)) which is being incorporated into the specification by reference (see below).

Applicants have incorporated the sequence of B7-1 (SEQ ID NO: 23) into the specification. Applicants respectfully submit that an appropriate location for the amino acid sequence of B7 antigen is on page 21, line 5. Therefore, Applicants have amended the specification at page 21, line 5, as set forth above.

A diskette containing B7-1 amino acid sequence of amino acids 1-216 as published on page 2717 by Freeman et al., 1989 (SEQ ID NO: 23) is enclosed. A paper copy of all the

Sequence Listing (i.e., SEQ ID NOs: 1-32) is also enclosed (Exhibit 1). A Declaration stating that the electronic copy of the Sequence Listings is identical to the paper copy is also enclosed (Exhibit 2)

Applicants respectfully submit that since the reference by Freeman et al, (1989) was incorporated by reference in the application (page 1, lines 12-16; page 34, lines 33-35), the incorporation of B7-1 amino acid sequence into the specification, does not introduce any new matter. A signed Declaration pursuant to MPEP 608.01(p) is also enclosed (Exhibit 3).

Accordingly, the amendments to claims 38 and 40 and new claims 43-44 do not introduce any new matter, and their entry is respectfully requested.

SEQUENCE COMPLIANCE

At page 2, paragraph 1 of the Office Action, the Examiner is requiring that each disclosed sequence have its own SEQ ID NO. In response, Applicants provide an updated sequence listing that includes amino acid sequence of the CTLA4 ligand B7 (SEQ ID NO: 23). Applicants also provide SEQ ID NOs. for all the primers and other sequences in the specification.

Paragraph 2

At page 2, paragraph 2 of the Office Action, the Examiner pointed that Claims 11-19 and 38-42 were being examined. Applicants have cancelled claims 11-19, 39, and 41-42 without prejudice. Applicants have also amended claims 38 and 40. Accordingly, claims 38 and 40 are now being examined.

RESTRICTION REQUIREMENT

At page 2, paragraph 3 of the Office Action, the Examiner acknowledged Applicants' election of Group 1 and the species neoplasia, i.e., claims 11, 12 and 38-40, with traverse, but maintained that the restriction requirement was proper, and made it final.

INVENTORSHIP

At page 2, paragraph 4, the Patent Office is requiring that Applicants verify the inventorship of the claimed methods in the subject application. Based on the pending claims, Dr. Peach is not an inventor. Applicants will file a change of inventorship upon indication of allowability of claims.

WRITTEN SUPPORT

At page 2, paragraph 5, the Office invited Applicants to verify that the instant claims have written support and enablement under 35 U.S.C 112, first paragraph, in U.S. Serial Nos. 08/008,898, filed January 2, 1993, and U.S. Serial No. 07/723,617, filed July 27, 1991, for methods of treating neoplasia with B7 and B7 fusion proteins as well as for B7-1 and B7-2.

In response, Applicants' respectfully point out that written support for instant claims may be found in U.S. Serial No. 7/723,617 at the following:

1) At page 7, line 31, U.S. Ser. No. 7/723,617 states "Also included in the invention is a method for regulating T cell interactions with other cells by inhibiting the interaction of CTLA4-positive T cells with B7 positive cells by reacting the T cells with ligands for the CTLA4 receptor. The ligands include B7Ig fusion protein, a monoclonal antibody reactive with CTLA4 receptor, and antibody fragments."

2) At page 25, lines 24-28, U.S. Ser. No. 7/723,617 "Regulation of CTLA4-positive T cell interactions with B7 positive cells, including B cells, by the methods of the invention may thus be used to treat pathological conditions such as autoimmunity, transplantation, infectious diseases and neoplasia."

3) Additionally, U.S. Ser. No. 7/723,617 at page 26, Example 1, discloses preparation of B7Ig fusion proteins.

Support for the instant claims may be found in U.S. Ser. No. 08/008,898 at the following:

1) At page 7, line 10, U.S. Ser. No. 08/008,898 states "Also provided are methods for using the CTLA4 fusion protein, B7Ig fusion protein, hybrid fusion proteins, and fragments and/or derivatives thereof, such as monoclonal antibodies reactive with CTLA4 and the B7 antigen, to regulate cellular interactions and immune responses."

2) At page 8, line 18, the above application states "Also included in the invention is a method for regulating T cell interactions with other cells by inhibiting the interaction of CTLA4-positive T cells with B7 positive cells by reacting the T cells with ligands for the CTLA4 receptor. The ligands include B7Ig fusion protein, a monoclonal antibody reactive with CTLA4 receptor, and antibody fragments."

3) At page 9, line 11, the above application states "Further, the present invention provides a method for blocking B7 interaction so as to regulate the immune response. This method comprises contacting lymphocytes with a CTLA4-binding molecule and an IL4-binding molecule."

4) At page 9, line 16, the above application states "Additionally, the present invention provides a method for regulating an immune response which comprises contacting B7-positive lymphocytes with a CTLA4-binding molecule and an IL4-binding molecule."

5) Additionally, at page 30, line 12, U.S. Serial No. 08/008,898 states "Regulation of CTLA4-positive T cell interactions with B7 positive cells, including B cells, by the methods of the invention may thus be used to treat pathological conditions such as autoimmunity, transplantation, infectious diseases and neoplasia."

INFORMATION DISCLOSURE STATEMENTS

At page 3, paragraph 6, the Office states that two Information Disclosure Statements (Paper No. 6, filed 8/3/00 and Paper No. 10, filed January 2, 01) were submitted, and appear to be duplicates of one another. However, Applicants' record show that only one Information Disclosure Statement was filed on July 28, 2000.

PRIORITY

At page 4, paragraph 7, the Office suggested updating the status of the priority documents. Accordingly, Applicants have amended the specification to update the status of the priority documents.

TITLE OF THE INVENTION

At page 4, paragraph 8, the Patent Office suggested amending the title of the invention to reflect currently pending claims. Accordingly, Applicants have amended the title of the subject application.

ABSTRACT OF THE DISCLOSURE

At page 4, paragraph 9, the Patent Office suggested amending the abstract of the disclosure to reflect pending claims. Accordingly, Applicants have amended the abstract.

FORMAL DRAWINGS

At page 4, paragraph 10, the Patent Office stated that formal drawings and photographs have been submitted which fail to comply with 37 CFR §1.84. Applicants will provide formal figures upon indication of allowability of claims.

TYPOGRAPHICAL ERRORS AND TRADEMARKS

At page , paragraph 11, the Patent Office required that the subject application be reviewed and all spelling, TRADEMARKS, and like errors corrected. Accordingly, Applicants have reviewed the application for spellings, TRADEMARKS, and other typographical errors, and amended the specification of the application to incorporate corrected spellings, TRADEMARKS, and other typographical errors. Applicants also provide herein a marked-up version indicating all the suggested changes.

REJECTION UNDER 35 U.S.C. 112, FIRST PARAGRAPH

The primary rejections under 35 U.S.C. 112, first paragraph are summarized as follows:

1. The term "inhibiting the interactions of CTLA4-positive T cells with B7 positive cells by contacting said T cells with a ligand for CTLA4 (i.e. B7 fusion protein)" is nonenabling for achieving the elected endpoint of treating neoplasia (Office Action, paragraph 13A) (discussed further in Section A herein);

2. The objective evidence of record does indicate that B7 molecules do not appear to achieve the therapeutic endpoints disclosed in the specification as filed (Office Action paragraph, 13B) (discussed further in Section B herein);

3. There is insufficient written description encompassing a "ligand for CTLA4" and "B7" (Office Action, paragraph 14) (discussed further in Section D herein).

4. The specification is not enabling for any B7 molecule (Office Action, paragraph 15) (discussed further in Section C herein);

At the outset, Applicants respectfully disagree with the rejections. A summary of Applicants' bases for traversal is as follows.

The first enablement rejection is improper. The claimed methods are directed to inhibiting functional CTLA4 positive T cell interactions with B7 positive cells. The methods of the invention work by enhancing T cell responses as claimed. For example, in the claimed method, a soluble B7 molecule, e.g., B7Ig, binds to CTLA4 receptor on activated T cells, with high affinity, thereby inhibiting the binding of the CTLA4 receptor on T cells to endogenous B7 antigen on B cells (claims 38 and 40). It is because of this inhibition of down-regulation of T cell mediated immune responses that we did not see significant inhibition of MLR with B7Ig even at higher concentrations as shown in Figure 9 of the subject application.

The second enablement rejection is improper. There is no reason to doubt Applicants' disclosure. In fact, the methods of the invention work as claimed. Applicants' in vitro data confirms the claimed methods using a soluble B7 molecule having the sequence of SEQ ID NO: 23 (i.e., B7-1) to bind CTLA4 receptor on T cells so as to inhibit B7/CTLA4 interaction. Accordingly, the rejection is rendered moot.

The written description rejection is improper. The pending claims are directed to **methods** of using soluble B7 having the sequence of SEQ ID NO: 23 (i.e., B7-1) to inhibit interaction of CTLA4 on activated T cells with endogenous B7 on B cells which were described in the original application to which the subject application claims priority. The Office cannot use later, post-filing date publications (Coyle et al., Nature Immunol.2;203-209 (2001)) to narrow the scope of the current claims by arguing lack of written description sufficient to describe other B7 molecules. However, in order to further the prosecution of the subject application and place the claims in condition for allowance, Applicants have amended the claims to recite the use of soluble B7 having the sequence of SEQ ID NO: 23 (i.e., B7-1). Accordingly, the rejection is rendered moot.

The third enablement rejection is improper because B7 is a known class of antigens. However, in order to further the prosecution of the subject application and place the claims in condition for allowance, applicants have followed the Examiner's suggestions and amended the claims to recite the use of a soluble B7 having the sequence of SEQ ID NO: 23 (i.e., B7-1). Accordingly, the rejection is rendered moot.

A detailed traversal may be found in Sections A, B, C and D as follows.

Section A. Enablement of Claims 11, 38 and 40

In paragraphs 12 and 13a, page 5 of the Office Action, the Patent Office rejected claims 11, 12 and 38-40 under U.S.C.112, first paragraph, allegedly as containing subject matter which was not described in the specification in such a way as to enable one skilled in the art to which it pertains, or with which it is most nearly connected, to make and/or use the invention.

The Office rejected claims 11, 12, and 38-40, alleging that with respect to the elected invention, claims 11, 12, and 38-40 are not enabled for achieving the elected end point of

treating neoplasia by "regulating CTLA4-positive T cell interactions with other cells comprising inhibiting the interaction of CTLA4-positive T cells with B7 positive cells by contacting a ligand for CTLA4 (i.e., B7 fusion protein)" or "regulating functional CTLA4 T cell interactions ---to interfere---" because B7 fusion protein acts as an adjuvant by enhancing T cell responses and not by inhibiting T cell responses as currently cited." (Office Action, page 5, lines 8-13). For support, the office cited Stumhoefel et al. (Cancer Research 59: 4964-4972 (1999)). (Office Action, page 5, line 18) and Figure 9 of the subject application. (Office Action, page 5, line 20).

The Law

Under 35 U.S.C. §112, first paragraph, Applicants need only provide enough information to one of ordinary skill in the art to practice the invention as claimed. In re Eynde, 480 F.2d 1364, 178 U.S.P.Q. 470 (C.C.P.A. 1973) ("That statutory requirement is fulfilled where one possessed of the knowledge had by one skilled in the art could use the invention given the specification disclosure without undue experimentation."). Furthermore, while "the scope of enablement varies inversely with the degree of unpredictability involved," Applicants are not required to disclose an example of *every* species covered by a claim. In re Angstadt, 537 F.2d 498, 502-503, 190 U.S.P.Q. 214 (C.C.P.A. 1976). "It is not necessary that patent applicant test all embodiments of his invention; what is necessary is that he provide disclosure sufficient to enable one skilled in the art to carry out the invention commensurate with the scope of his claims." Amgen, Inc. v. Chugai Pharmaceutical Co., Ltd. 927 F.2d 1200 (1991).

As discussed above, "[c]ompliance with §112, paragraph one, is to be judged as of the date the application is filed." In re Koller, 613 F.2d 819, 823 (C.C.P.A. 1980), *citing* In re Hogan, 559 F.2d 595 (C.C.P.A. 1977). Just as later discoveries cannot be used to support rejections under §§102 or 103, evidence of later discoveries cannot be used to

support a rejection under §112. In re Hogan, 559 F.2d 595, 194 U.S.P.Q. 527 (C.C.P.A. 1977). The CCPA, in In re Hogan, further stated:

> "As pioneers, if such they be, they would deserve broad claims to the broad concept. What were once referred to as 'basic inventions' have led to 'basic patent,' which amounted to real incentives, not only to invention and its disclosure, but to it prompt, early disclosure. If later states of the art could be employed as a basis for rejection under 35 U.S.C. §112, the opportunity for obtaining a basic patent upon early disclosure of pioneer inventions would be abolished." Id. at 606.

Applicants' Claimed Methods Are Fully Enabled

It is well known in the art that binding of B7 antigen to CTLA4 on activated T cells down-regulates immune responses (Chambers, A. and Allison, J.P. *Current Opinion in Immunol.* (1997) 9:396-404)). In the instant claims, soluble B7 molecule, e.g., B7Ig, binds to CTLA4 on activated T cells, with high affinity, thereby inhibiting binding of CTLA4 on T cells to endogenous B7 antigen on B cells. This inhibition of binding of CTLA4 on T cells to endogenous B7 antigen on B cells in turn results in inhibition of down-regulation of T cell mediated immune responses, such as lymphoproliferative responses as shown in Figure 9 of the subject application.

Further, Sturmhoefel et al., (Cancer Research 4964-4972 (1999)) as cited by the Examiner does not provide support for the Examiner's basis for doubting the enablement of Applicants' methods. Sturmhoefel et al. actually confirms Applicants' invention.

Sturmhoefel et al. discloses use of B7Ig fusion protein in therapy of established tumors in three mice models and as a vaccine adjuvant. In all tumor models, B7-1-Ig and B7-2-Ig had similar antitumor activity. The antitumor activity of B7-Ig was independent of IFN-γ activity. The above-cited reference states "Blocking the interaction of B7 with CTLA4,

thereby preventing negative signals triggered by CTLA4, has also been used as an approach to enhance antitumor activity." (page 4964, column 2, paragraph 1)

Sturmhoefel et al., states that " several hypotheses can be proposed for the mechanism by which B7-IgG fusion proteins assist the generation of novel antitumor responses or enhance existing antitumor responses. B7.1-IgG and B7.2-IgG can bind both CD28 and CTLA-4. Therefore, B7-IgG has the potential to enhance costimulatory effects through CD28 as well as to prevent negative signals triggered through CTLA4." (page 4970, column 2, paragraph2)

Sturmhoefel et al., further states "In addition, in therapeutic settings, soluble B7-IgG may bind with high affinity to CTLA4 on activated T cells to block its negative signal, thereby enhancing the activity of tumor specific T cells or preventing their down-regulation." (page 4971, column 1, paragraph 1)

Applicants respectfully request that in view of the amendments to the claims and the remarks, that the Examiner reconsider and withdraw the outstanding rejections of the claims under 35 U.S.C. §112, first paragraph for enablement.

Section B. Enablement of claims 11, 38 and 40

In paragraph 13b, at page 5 of the Office Action, the Patent Office rejected claims 11, 38 and 40 under 35 U.S.C. 112, first paragraph, asserting that "in vitro and animal model studies have not correlated well in vivo clinical trial results in patients. *** There is insufficient objective evidence that accurately reflects the relative efficacy of the claimed method to inhibit functional CTLA4;T cell interactions to regulate cellular interactions and immune responses in vitro by administering B7 or B7 fusion proteins, commensurate in scope with the therapeutic methods encompassed by the claimed invention." Furthermore, the Patent Office alleges "based upon the objective evidence disclosed in

the instant specification, and in the art, the skilled artisan could not predict the efficacy or enablement of B7 or B7 fusion proteins to inhibit CTLA4:T cell interactions by inhibiting T cell functions in the targeted diseases or patients encompassed by the claimed methods." (Office Action, pages 6-8)

Applicants respectfully disagree with this ground of rejection and submit that the specification is adequately enabled for the claimed methods for the reasons discussed above in Section A. Further, others provide support that the claimed methods are effective *in vivo*. For example, as discussed above, Sturmhoefel et al. (Cancer Research 4964-4972 (1999)), utilizes B7-1 IgG and B7-2 IgG in *in vivo* studies to show that it promotes antitumor immune responses. Specifically, Sturmhoefel et al. states "B7-IgG fusion proteins appear to be effective antitumor agents that seem to be safe and can readily be administered in the clinic and manufactured. Their potency in stimulating immune responses and cure multiple murine models suggest clinical potential as an adjuvant and therapy for oncology and for other clinical indications."

Further, Applicants wish to point out that the Examiner's references do not establish that Applicants' data does not correlate with *in vivo* methods. The references cited by the Patent Office were published after the effective date of the subject application, and ***none*** refutes the correlation of Applicants' *in vitro* data with *in vivo* results.

(a) Kahan

Kahan reviews immunosuppressive therapy available in 1992. The standard clinical therapy at that time was Cyclosporin A (CsA) with researchers trying to combine CsA therapy with other agents, such as other drugs or antibodies, to improve treatment efficacy. Kahan describes the outcomes of various CsA + other existing agent treatments; none of which worked very well. Kahan then goes on to describe "second generation

mAbs", chimeric mAbs and humanized mAbs and reviews the outcomes of some experiments with these newer mAbs.

Kahan does not mention B7 or CTLA4 or the claimed methods of the invention. CD28 is mentioned in the sentence on page 555, 2nd column, "However, a major limitation may be the failure of CsA to inhibit lymphocyte activation via the CD28 surface marker..."

Nowhere in the article does Kahan suggest that soluble B7 molecules shown to be effective *in vitro* could not be useful for clinical exploration. The alleged lack of correlation, at the time Kahan was written, between in vitro immune assays and in vivo immunosuppressive efficacy, is limited to the then known methods and reagents, which at that time (1992), hindered the development of efficacious immunosuppressive regimens.

(b) Blazar et al. (J. Immnuol. 167:3250-3259(1997))

The Patent Office summarizes Blazar et al., as disclosing that "anti-CD80 or anti CD86 antibodies were ineffective in preventing T cell CD28-mediated GVHD lethality; that each antibody was partially effective in CD4-mediated GVHD lethality[,] and that the combination of anti-CD80 and anti-CD86 antibodies were[as] effective in preventing GVHD lethality in murine experimental models." (Office Action, page 6).

As acknowledged by the Patent Office, Blazar et al., shows that in a murine model, infusion of anti-B7-1 and anti-B7-2 monoclonal antibodies inhibits GVHD lethality and therefore, indirectly supports Applicants' position. Contrary to the Office's interpretation, Blazar et al., do not suggest that soluble B7 molecules shown to be effective *in vitro* would <u>not</u> be useful for clinical exploration.

(c) Perrin et al.(J. Neuroimmunol. 65:31-39(1996))

The Patent Office characterizes Perrin et al. as finding that "in contrast to the effective treatment of disease with CTLA4-Ig; anti-CD80 (B7-1) attenuated the first clinical disease episode but not the relapse, anti-CD86 (B7-2) had no significant effect on the course of disease, and the combined treatment with anti-CD80 plus anti-CD86 resulted in the exacerbation of disease." (Office Action. Page 6, paragraph 13).

Perrin et al. used an Experimental Allergic Encephalomyelitis ("EAE") model in mice. With respect to the results obtained using monoclonal antibodies directed to CD80 and CD86, Perrin et al. offer several possible explanations – none of which were proven or rejected during the course of their work reported in the subject article or theresfter. Specifically, Perrin et al. state:

> "In these studies, CD80-specific mAb treatment following immunization with MBP resulted in alleviation of clinical disease, while anti-CD80 plus anti-CD86 treatment resulted in exacerbation of disease. The differential effects of anti-CD80 plus anti-CD86 and CTLA4-Ig treatment on disease progression ***suggest*** either the presence of a third B7-like molecule that has a role during the inductive limb of this immune response or signal transduction through the B7 receptors themselves. ***Another possibility*** is that these reagents may differ in their ability to interfere with a regulatory signal provided through CTLA-4 molecules. To address the possibility of signal transduction through the B7 receptors themselves, we attempted to treat the animals with anti-CD80 Fab plus anti-CD86 Fab on day 2 post immunization. This treatment did not affect the course of disease. Given the rapid excretion of Fab fragments via the kidneys, ***the interpretation of this result is not clear***. Furthermore, ***results obtained after the prolonged administration of Fab fragments and those obtained from a single injection of intact antibody molecules could not be legitimately compared. Finally, it is possible that an interaction between the anti-B7 reagents and PT has resulted in disease exacerbation. In an active model of EAE which does not require PT treatment, injection of anti-CD80 plus anti-CD86 on day 2 post immunization resulted in decreased incidence and severity of clinical disease rather than the exacerbated disease observed in the present study***." Id. at 37.

Thus, the findings of Perrin et al. are, at best, inconclusive with respect to efficacy of the anti-CD80 and anti-CD86 molecules in their mouse models. But the fact remains, that those of skill in the art, including Perrin et al., are obtaining *in vivo* alleviation of disease using anti-B7 antibodies. Morever, contrary to Office's interpretation, Perrin et al., does not suggest that the use of soluble B7 molecule in Applicants claimed methods, shown to be effective *in vitro* would not be useful for clinical exploration.

(d) Yi-qun et al.

The Patent Office further characterizes Yi-qun et al., as demonstrating that "[i]t is clear that inhibition of T cell responses to soluble antigens will require the blocking of both B7-2 and B7-1 to be effective. More, important it is unlikely that ongoing T cell response will be susceptible to inhibition by anti-B7 reagents, for example in autoimmune diseases." (Office Action, page 7, Paragraph 13).

This is again a mischaracterization of what is disclosed by the reference. Yi-qun et al., show that anti-CD80 and anti-CD86 are both effective individually, but may not be as effective as when used in combination.

Section C. Written Description Rejections

In paragraph 14, at page 11 of the Office Action, the Patent Office rejected claims 11, 12 and 38-40 under 35 U.S.C. 112, first paragraph, alleging that the specification does not contain a written description of the claimed invention, in that the disclosure does not reasonably convey to one skilled in the relevant art that the inventors had possession of the claimed invention at the time the application was filed (Office Action, page 8).

Applicants respectfully disagree with the grounds of this rejection and submit that the specification contains an adequate written description of the invention claimed in claims 11, 12 and 38-40.

However, in order to further the prosecution of the subject application, Applicants have canceled claims 11, 12 and 39 and amended claims 38 and 40 in accord with the Examiner's suggestion, without prejudice to pursue the substance of these claims in a related application.

Section D. Enablement of Claims 11, 38 and 40

In paragraph 15, at page 12 of the Office Action, the Patent Office rejected claims 11, 12 and 38-40 under 35 U.S.C. 112, first paragraph, alleging that the specification while being enabling for B7-1 and B7-2 (and the non-elected CTLA4-specific antibody) as the claimed CTLA4 ligand and B7 protein or B7 fusion protein, does not reasonably provide enablement for any "CTLA4 ligand" or "B7 protein or B7 fusion protein." The Office Action further states "specification does not enable any person skilled in the art to which it pertains, or with which it is most clearly connected, to make and use the invention commensurate in scope with these claims." (Office Action, page 11).

Applicants respectfully disagree. However, in order to further the prosecution of the subject application, applicants have amended the claims in accordance with the Examiner's suggestions to recite the use of B7 having the sequence of SEQ ID NO: 23 (i.e., B7-1), without prejudice.

REJECTION UNDER 35 U.S.C 112, SECOND PARAGRAPH

Paragraph 16

The Office rejected claims 11, 12 and 38-40 under 35 U.S.C. 112, second paragraph, allegedly as being indefinite for failing to particularly point out and distinctly claim the subject matter which applicant regards as the invention.

Specifically, the Patent Office noted:

A) "Claims 11, 12, and 38-40 are indefinite in its recitation of "regulating CTLA4-positive T cell interactions with other cells comprising inhibiting the interaction of CTLA4-positive T cells with B7-positive cells by contacting said T cell with a ligand for CTLA4 (i.e., B7 fusion protein). The recitation "regulating" is indefinite because it is ambiguous as to the nature, direction (positive or negative)." (Office Action, page 13)

B) "Claims 11, 12 and 38-40 are indefinite in the recitation of "B7" in that they only describe the products of interest by an arbitrary name. While the name itself may have some notion of the activity of the protein, there is nothing in the claims which distinctly claims the protein and fusion proteins thereof." (Office Action, page 13).

With respect to the phrase "regulating CTLA4-positive T cell interactions with other cells----" and "regulating functional CTLA4 T cell interactions," Applicants point out that claims further recites "inhibiting", thus, the claims are not idefinite. Applicants reassert that a T cell response may either be up-regulated or down-regulated. Both up-regulation (i.e., induction or stimulation) and down-regulation (i.e., inhibition) are disclosed in the specification (*See* Specification, page 6, lines 3-8; page 9, 25-35; page 12, lines 15-16; page 14, lines 1-34; page 17, lines 25-35;). Again, the crux of the invention is the

interaction of B7 on B cells and CTLA4 on T cells and the interruption of this interaction. Thus, the recitation of the "regulation of T cell interactions" is sufficiently definite and clear.

With respect to recitation of "B7", Applicants' as discussed earlier herein, maintain that "B7" is not an arbitrary protein name, but rather a *class* of antigens known to those of skill in the art.

However, to further the prosecution of the subject application, Applicants have amended claims 38, and 40 such that the term "regulating" has been changed to the term "inhibiting" and soluble B7 has the sequence of SEQ ID NO: 23.

In view of the amendments to claims 38, and 40, Applicants request that the Examiner withdraw the rejection of the instant claims under 35 U.S.C. 112, second paragraph.

REJECTION UNDER 35 U.S.C. 102(e)

Paragraphs 17-19

The Office rejected claims 11-12 and 38-40 under 35 U.S.C. 102(e) as allegedly anticipated by Linsley et al. (U.S. Patent No. 5,580,756).

Applicants respectfully disagree for the reasons provided below.

The Federal Circuit has held that an invention is anticipated only if all elements of the claimed invention are present in a product or process disclosed, expressly or inherently, in a single prior art reference.[1]

U.S. Patent No. 5,580,756 identifies the B7 antigen as a ligand that is reactive with the CD28 antigen on T cells. This patent further discloses methods for using B7 antigen, its fragments and derivatives, and the CD28 receptor, its fragments and derivatives, as well as other molecules reactive with B7 antigen and/or CD28 receptor, to regulate CD28 positive T cell responses, and immune responses mediated by T cells.

U.S. Patent No. 5,580,756 doe not describe or suggest CTLA4 receptor or the instant claims, i.e., methods for using B7 antigen, its fragments and derivatives, to regulate CTLA4 positive T cell responses, and does not disclose B7 as a ligand that is reactive with CTLA4 antigen. This was Applicants' pioneering discovery.

The '756 patent cited by the Examiner does not disclose each element of the methods claimed in the present application, as required by 102(e). Since the '756 patent does not describe CTLA4, it cannot anticipate the claimed methods.

CONCLUSION

Applicants believe that all grounds for rejection of the claims have been successfully overcome and that the claims are now in condition for allowance. Withdrawal of the Examiner's remaining rejections is requested and prompt allowance of the claims is solicited. If any issues remain in connection with the claims, the Examiner is encouraged to contact the undersigned by telephone to discuss the same.

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[1] RCA Corp. v. Applied Digital Data Sys., Inc., 730 F.2d 1440, 221 USPQ 385, 388 (Fed. Cir. 1984), *cert. dismissed sub nom.* Hazeltine Corp. v. RCA Corp., 468 U.S. 1228.

Attached hereto is a marked-up version of the changes made to the application by this Amendment.

Although no additional fee, except for the Petition Fee enclosed herewith, should be required, the Commissioner is hereby authorized to charge any additional fee, or credit any over-payment, to Deposit Account No. 50-0306.

Respectfully submitted,

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VERSION WITH MARKINGS TO SHOW CHANGES MADE

In the Title:

In accordance with 37 C.F.R. §§1.121(b)(1)(i)-(ii), please amend the title of the application on the title page and page 1, to read as follows:

-- [CTL4 MOLECULES AND IL4-BINDING MOLECULES AND USES THEREOF] METHODS FOR INHIBITING INTERACTIONS BETWEEN CTLA4-POSITIVE T CELLS AND B7 POSITIVE CELLS --

In the Specification:

Please amend the specification at page 1, lines 6-10, to read as follows:

--This application is a divisional application of U.S. Serial No. 08/228,208, filed April 15, 1994, now U.S. Patent No. 6,090,914, which is a continuation-in-part of U.S. Serial No. 08/008,898, filed January 22, 1993, now U.S. Patent No. 5,776,197, which is a continuation-in-part of U.S. Serial No. 723,617, filed July 27, 1991, now abandoned, the contents of all of which are incorporated by reference in their entirety into this application. --.

Please amend the specification at page 21, line 5, please insert :

-- The predicted amino acid sequence for amino acids 1-216 (SEQ ID NO; 23) of the B7 antigen, isolated by Freeman et al., (*Supra*) is:

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Gly Leu Ser His Phe Cys Ser Gly Val Ile His Val Thr Lys Glu Val
 1               5                  10                  15

Lys Glu Val Ala Thr Leu Ser Cys Gly His Asn Val Ser Val Glu Glu
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20 25 30

Leu Ala Gln Thr Arg Ile Tyr Trp Gln Lys Glu Lys Lys Met Val Leu

35 40 45

Thr Met Met Ser Gly Asp Met Asn Ile Trp Pro Glu Tyr Lys Asn Arg

50 55 60

Thr Ile Phe Asp Ile Thr Asn Asn Leu Ser Ile Val Ile Leu Ala Leu

65 70 75 80

Arg Pro Ser Asp Glu Gly Thr Tyr Glu Cys Val Val Leu Lys Tyr Glu

85 90 95

Lys Asp Ala Phe Lys Arg Glu His Leu Ala Glu Val Thr Leu Ser Val

100 105 110

Lys Ala Asp Phe Pro Thr Pro Ser Ile Ser Asp Phe Glu Ile Pro Thr

115 120 125

Ser Asn Ile Arg Arg Ile Ile Cys Ser Thr Ser Gly Gly Phe Pro Glu

130 135 140

Pro His Leu Ser Trp Leu Glu Asn Gly Glu Glu Leu Asn Ala Ile Asn

145 150 155 160

Thr Thr Val Ser Gln Asp Pro Glu Thr Glu Leu Tyr Ala Val Ser Ser

165 170 175

Lys Leu Asp Phe Asn Met Thr Thr Asn His Ser Phe Met Cys Leu Ile
180 185 190

Lys Tyr Gly His Leu Arg Val Asn Gln Thr Phe Asn Trp Asn Thr Thr
195 200 205

Lys Gln Glu His Phe Pro Asp Asn --
210 215

Please amend the specification at page 21, line 18-33, to read as follows:

-- Because the expression of CTLA4 receptor protein in human lymphoid cells has not been previously reported, it was necessary to locate a source of CTLA4 mRNA. PCR cDNA made from the total cellular RNA of several human leukemia cell lines was screened, using as primers, oligonucleotides from the published sequence of the CTLA4 gene (Dariavach et al., supra). Of the cDNA tested, H38 cells (an HTLV II-associated leukemia line) provided the best yield of PCR products having the expected size. Since a signal peptide for CTLA4 was not identified in the CTLA4 gene, the N terminus of the predicted sequence of CTLA4 was fused to the signal peptide of oncostatin M (Malik et al., Molec. and Cell. Biol. 9:2847 (1989)) in two steps using oligonucleotides as described in the Examples, infra. The product of the PCR reaction was ligated with cDNA encoding the amino acid sequences corresponding to the hinge, CH2 and CH3 regions of Ig C 1 into an expression vector, such as CDM8 or πLN. --.

Please amend the specification at page 22, line 32 to page 23, line 10, to read as follow:

-- The clones containing DNA encoding fusion constructs obtained as described above are then transfected into suitable host cells for expression. Depending on the host cell used, transfection is performed using standard techniques appropriate to such cells. For example, transfection into mammalian cells is [ ]accomplished using DEAE-[d]Dextran™ mediated transfection, $CaPO[0]_4$ co-precipitation, lipofection, electroporation, or protoplast fusion,

and other methods known in the art including: lysozyme fusion or erythrocyte fusion, scraping, direct uptake, osmotic or sucrose shock, direct microinjection, indirect microinjection such as via erythrocyte-mediated techniques, and/or by subjecting host cells to electric currents. The above list of transfection techniques is not considered to be exhaustive, as other procedures for introducing genetic information into cells will no doubt be developed. --.

Please amend the specification at page 33, lines 26 to page 34, line 34, to read as follows:
-- For example, the present invention affects the transplant antigen-specific T cells, thus inducing donor-specific and antigen-specific tolerance. The binding of CD28 by its ligand, B7/BB1 (B7), during T cell receptor engagement is critical for proper T cell signaling in some systems (M. K. Jenkins, P. S. Taylor, S. D. Norton, K. B. Urdahl, J. Immunol. 147:2461 (1991); C. H. June, J. A. Ledbetter, P. S. Linsley, C. B. Thompson, Immunol. Today 11:211 (1990); H. Reiser, G. J. Freeman, Z. Razi-Wolf, C. D. Gimmi, B. Benacerraf, L. M. Nadler, Proc. Natl. Acad. Sci. U.S.A. 89:271 (1992); N. K. Daml[I]e, K. Klussman, P. S. Linsley, A. Aruffo, J. Immunol. 148:1985 (1992)). --.

Please amend the specification at page 37, lines 16-31, to read as follows:
-- Cell Culture and Transfections. COS (monkey kidney cells) were transfected with expression plasmids expressing CD28 and B7 using a modification of the protocol of Seed and Aruffo (Proc. Natl. Acad. Sci. 84:3365 (1987)), incorporated by reference herein. Cells were seeded at 10^6 per 10 cm diameter culture dish 18-24 h before transfection. Plasmid DNA was added (approximately 15 μg/dish) in a volume of 5 mls of serum-free DMEM™ containing 0.1 mM chloroquine and 600 g/ml DEAE Dextran™, and cells were incubated for 3-3.5 h at 37 C. Transfected cells were then briefly treated (approximately 2 min) with 10% dimethyl sulfoxide in PBS and incubated at 37°C for 16-24 h in DMEM™ containing 10% FCS. At 24 h after transfection, culture medium was removed and replaced with serum-free DMEM™ (6 ml/dish). Incubation was continued for 3 days at 37°C, at which time the spent medium was collected and fresh serum-free medium was added. After an

additional 3 days at 37°C, the spent medium was again collected and cells were discarded. --.

Please amend the specification at page 37, line 33 to page 38, line 13, to read as follows:

-- CHO cells expressing CD28, CD5 or B7 were isolated as described by Linsley et al., (1991) supra, as follows: Briefly, stable transfectants expressing CD28, CD5, or B7, were isolated following cotransfection of dihydrofolate reductase-deficient Chinese hamster ovary ($dhfr^-$ CHO) cells with a mixture of the appropriate expression plasmid and the selectable marker, pSV2dhfr (Linsley et al., Proc. Natl. Acad. Sci. USA 87:5031 (1990)), incorporated by reference herein. Transfectants were then grown in increasing concentrations of methotrexate to a final level of 1μM and were maintained in DMEM™ supplemented with 10% fetal bovine serum (FBS), 0.2 mM proline and 1 μM methotrexate. CHO lines expressing high levels of CD28 ($CD28^+$ CHO) or B7 ($B7^+$ CHO) were isolated by multiple rounds of fluorescence-activated cell sorting ($FACS^R$) following indirect immunostaining with mAbs 9.3 or BB-1. Amplified CHO cells negative for surface expression of CD28 or B7 ($dhfr^+$ CHO) were also isolated by $FACS^R$ from CD28-transfected populations. --.

Please amend the specification at page 38, lines 15-28, to read as follows:

-- Immunostaining and $FACS^R$ Analysis. Transfected CHO or COS cells or activated T cells were analyzed by indirect immunostaining. Before staining, CHO cells were removed from their culture vessels by incubation in PBS containing 10 mM EDTA. Cells were first incubated with murine mAbs 9.3 (Hansen et al., Immunogenetics 10:247 (1980)) or BB-1 (Yokochi et al., J. Immunol. 128:823 (1981)), or with Ig fusion proteins (all at 10 μg/ml in DMEM™ containing 10% FCS) for 1-2 h at 4°C. Cells were then washed, and incubated for an additional 0.5-2h at 4°C with a FITC-conjugated second step reagent (goat anti-mouse Ig serum for murine mAbs, or goat anti-human Ig C serum for fusion proteins (Tago, Inc., Burlingame, CA)). Fluorescence was analyzed on a FACS IV^R cell sorter

(Becton Dickinson and CO., Mountain View, CA) equipped with a four decade logarithmic amplifier. --.

Please amend the specification at page 40, lines 29-32, to read as follows:

-- Expression plasmids, CDM8, containing CTLA4Ig were then transfected into COS cells using DEAE/Dextran™ transfection by modification (Linsley et al., 1991, supra) of the protocol described by Seed and Aruffo, 1987, supra. --.

Please amend the specification at page 41, lines 8-13, to read as follows:

-- A preferred stable transfectant, expressing CTLA4Ig, designated Chinese Hamster Ovary Cell Line, CTLA4Ig-24, was made by screening B7 positive CHO cell lines for B7 binding activity in the medium using immunostaining. Transfectants were maintained in DMEM™ supplemented with 10% fetal bovine serum (FBS), 0.2 mM proline and 1 μM methotrexate. --.

Please amend the specification at page 42, lines 5-13, to read as follows:

-- To reconstruct DNA encoding the amino acid sequence corresponding to the full length human CTLA4 gene, cDNA encoding amino acids corresponding to a fragment of the transmembrane and cytoplasmic domains of CTLA4 was cloned by PCR and then joined with cDNA encoding amino acids corresponding to a fragment from CTLA4Ig that corresponded to the oncostatin M signal peptide fused to the N-terminus of CTLA4. Procedures for PCR, and cell culture and transfections were as described above in Example 1 using COS cells and DEAE-[d]Dextran™ transfection. --.

Please amend the specification at page 44, lines 1-12, to read as follows:

-- Immunostaining and FACS® Analysis. Prior to staining, COS or CHO cells were removed from their culture vessels by incubation in PBS containing 10 mM EDTA. Cells were first incubated with mAbs or Ig fusion proteins at 10 μg/ml in DMEM™ containing 10% FBS for 1-2 hr at 4°[ ]C. Cells were then washed, and incubated for an additional 0.5-

2 hrs at 4°[ ]C with FITC-conjugated goat anti-mouse immunoglobulin or with FITC-conjugated goat anti-human Ig Cγ serum (both from Tago, Burlingame, CA). When binding of both mAbs and Ig fusion proteins were measured in the same experiment, FITC-conjugated anti-mouse and anti-human second step reagents were mixed together before use. Fluorescence on a total of 10,000 cells was then analyzed by FACS^{R}. --.

Please amend the specification at page 44, lines 14-30, to read as follows:

-- Peripheral Blood Lymphocyte Separation and Stimulation. Peripheral blood lymphocytes (PBLs) were isolated by centrifugation through Lymphocyte Separation Medium^{TM} (Litton Bionetics, Kensington, MD). Alloreactive T cells were isolated by stimulation of PBL in a primary mixed lymphocyte reaction (MLR). PBL were cultured at 10^6/ml irradiated (5000 rad) T51 LCL. EBV-transformed lymphoblastoid cell lines (LCL), PM (Bristol-Myers Squibb Co.) and T51 (Bristol-Myers Squibb Co.) were maintained in RPMI^{TM} supplemented with 10% FBS. After 6 days, alloreactive "blasts" cells were cryopreserved. Secondary MLR were conducted by culturing thawed alloreactive blasts together with fresh irradiated T51 LCL in the presence and absence of mAbs and Ig fusion proteins. Cells were cultured in 96 well flat bottom plates (4×10^4 alloreactive blasts and 1×10^4 irradiated T51 LCL cells/well, in a volume of 0.2 ml) in RPMI^{TM} containing 10% FBS. Cellular proliferation of quadruplicate cultures was measured by uptake of [^{3}H]-thymidine during the last 6 hours of a 2-3 day culture. --.

Please amend the specification at page 44, line 32 to page 45, line 2, to read as follows:

-- PHA-activated T cells were prepared by culturing PBLs with 1 μg/ml PHA (Wellcome, Charlotte, NC) for five days, and one day in medium lacking PHA. Viable cells were collected by sedimentation through Lymphocyte Separation Medium^{TM} before use. Cells were stimulated with mAbs or transfected CHO cells for 4-6 hr at 37°C, collected by centrifugation and used to prepare RNA. ---.

Please amend the specification at page 45, lines 10-18, to read as follows:

-- B cells were also purified from peripheral blood by panning as described by Wysocki and Sato, Proc. Natl. Acad. Sci. 75:2844 (1978), incorporated by reference herein, using anti-CD19 mAb 4G9. To measure T_h-induced Ig production, 10^6 $CD4^+$ T cells were mixed with 10^6 $CD19^+$ B cells in 1 ml of RPMI™ containing 10% FBS. Following culture for 6 days at 37°C, production of human IgM was measured in the culture supernatants using solid phase ELISA as described by Volkman et al., Proc. Natl. Acad. Sci. USA 78:2528 (1981), incorporated by reference herein. --.

Please amend the specification at page 46, lines 8-16, to read as follows:

-- Immunoprecipitation Analysis and SDS PAGE. Cells were surface-labeled with ^{125}I and subjected to immunoprecipitation analysis. Briefly, PHA-activated T cells were surface-labeled with ^{125}I using lactoperoxidase and H_2O_2 as described by Vitetta et al., J. Exp. Med. 134:242 (1971), incorporated by reference herein. SDS-PAGE chromatography was performed on linear acrylamide gradients gels with stacking gels of 5% acrylamide. Gels were stained with Coomassie Blue, destained, and photographed or dried and exposed to X ray film (Kodak™ XAR-5). ---.

Please amend the specification at page 46, lines 18-28, to read as follows:

-- Binding Assays. B7Ig was labeled with ^{125}I to a specific activity of approximately 2×10^6 cpm/pmole. Ninety-six well plastic dishes were coated for 16-24 hrs with a solution containing CTLA4Ig (0.5 μg in a volume of 0.05 ml of 10 mM Tris, pH 8). Wells were blocked with binding buffer (DMEM™ containing 50 mM BES (Sigma Chemical Co.), pH 6.8, 0.1% BAS, and 10% FCS) before addition of a solution (0.09 ml) containing ^{125}I B7Ig (approximately 5×10^5 cpm) in the presence or absence of competitor. Following incubation for 2-3 hrs at 23°[ ]C, wells were washed once with binding buffer, and four times with PBS. Bound radioactivity was then solubilized by addition of 0.5N NaOH, and quantified by gamma counting. --.

Please amend the specification at page 61, lines 21-24, to read as follows:

-- Cells were maintained in DMEM™ supplemented with 10% fetal bovine serum (FBS), 0.2mM proline, and 1μM methotrexate. COS cells were grown in DMEM™ supplemented with 10% FBS. CTLA4Ig was prepared in CHO cells as previously described (Example 2). --.

Please amend the specification at page 62, lines 1-4, to read as follows:

-- Six mutants were prepared which encoded substitutions to alanine in the highly conserved hexapeptide 98MYPPPY103 (SEQ ID 24) forming part of the putative CDR3-like domain (Figures 17 and 22) (Ho et al., 1989, supra.). These mutants are described in Table II. --.

Please amend the specification at page 62, lines 6-9, to read as follows:

-- In addition, two mutants encoding the residues P103A and Y104A (MYPPAY (SEQ ID NO: 32) and MYPPPA (SEQ ID NO: 33), respectively) from the CD28Ig 99MYPPPY104 hexapeptide using CD28Ig as a template were also prepared by the same method. These mutants are also described in Table II. --.

Please amend the specification at page 62, lines 18-20, to read as follows:

-- These primers encoded the following sequences:

CDM8FP:5'-AATACGACTCACTATAGG (SEQ ID NO: 15)

CDM8RP:5'-CACCACACTGTATTAACC (SEQ ID NO: 16) --.

Please amend specification at page 66, lines 11-16, to read as follows:

-- *Immunoprecipitation and Western blot analysis.* CTLA4/CD28Ig hybrid fusion proteins present in culture media were adsorbed to protein A-Sepharose™ by overnight incubation at 4°C. The beads were washed with PBS containing 0.1% Nonidet-P40 (NP40) then SDS PAGE sample buffer was added and the eluted protein was loaded onto an SDS polyacrylamide gel. --.

Please amend the specification at page 77, lines 9-22, to read as follows:

-- CTLA4Ig MUTANT FUSION PROTEIN

AYPPPY (SEQ ID NO: 25)	+++	+++	+++	-
MAPPPY (SEQ ID NO: 26)	++	+	++	-
MYAPPY (SEQ ID NO: 27)	+	-	+	-
MYPAPY (SEQ ID NO: 28)	+++	++++++	+++	-
MYPPAY (SEQ ID NO: 29)	+++	-	+	-
MYPPPA (SEQ ID NO: 30)	+++	++	+++	-
AAPPPY (SEQ ID NO: 31)	+	++	+++	-

CD28Ig MUTANT FUSION PROTEIN

MYPPAY (SEQ ID NO: 32)	-	-	-	-
MYPPPA (SEQ ID NO: 33)	-	-	-	+ --.

In the Claims:

Please amend the claims as follows:

--38. (Amended) A method for [regulating] inhibiting functional CTLA4 positive T cell interactions with B7 positive cells comprising contacting the CTLA positive cells with a ligand for CTLA4, in an amount effective to interfere with reaction of endogenous B7 antigen with the CTLA4 positive cells, wherein the ligand for CTLA4 is a soluble B7 comprising the extracellular domain of B7 having the sequence disclosed in SEQ ID NO:23. --

--40. (Amended) The method of claim 3[9]8, wherein the soluble B7 protein is the extracellular domain of B7 joined to at least a portion of an immunoglobulin molecule [a B7Ig fusion protein]. --.

Please add new claims 43-44 as follows:

--43. (new) The method of claim 38, wherein the extracellular domain of B7 begins at position 1 and ends at position 215 of SEQ ID NO.: 23.

--44. (new) The method of claim 40, wherein the soluble B7 protein is a B7Ig fusion protein.

In the Abstract:

Please amend the abstract of the application as follows:

-- [The invention identifies the CTLA4 receptor as a ligand for the B7 antigen. The complete amino acid sequence encoding human CTLA4 receptor gene is provided. Methods are provided for expressing CTLA4 as an immunoglobulin fusion protein, for preparing hybrid CTLA4 fusion proteins, and for using the soluble fusion proteins, fragments and derivatives thereof, including monoclonal antibodies reactive with B7 and CTLA4, to regulate T cell interactions and immune responses mediated by such interactions.] This invention provides a method for regulating T cell interactions with B7 positive cells. Methods are provided for using B7 antigen, its fragments and derivatives reactive with CTLA4 receptor, to regulate CTLA4 positive T cell responses, and immune responses mediated by T cells. --.